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MAJOR ARTICLE

Diagnostic Approach for Classic Compared With Localized Whipple Disease

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Background. Whipple disease (WD), a rare systemic infection caused by *Tropheryma whipplei,* can be a diagnostic challenge due to its variable presentation. The role of *T. whipplei* polymerase chain reaction (PCR) is unclear as small bowel biopsy with Periodic acid-Schiff (PAS) staining remains the diagnostic gold standard. Individualized diagnostic approaches based on variable clinical manifestations are underutilized. We investigated the methodologies employed at our institution to diagnose WD.

Methods. We retrospectively collected all cases of WD diagnosed from 1994 to 2016. Microbiology laboratory and anatomic pathology databases were queried. Case characteristics and disease clinical phenotypes (classical, localized WD arthritis, and localized central nervous system [CNS] disease) were described. The diagnostic approach and testing yield were analyzed and reported.

Results. Thirty-three cases of WD were diagnosed (18 classic WD [CWD], 9 localized WD arthritis [LWD], 6 CNS WD). Misdiagnosis and delay in diagnosis were frequent. Diagnostic approach and test yield differed by classical vs localized WD involvement. Small bowel tissue biopsy PAS stain/PCR was overwhelmingly positive (86%/92%) in CWD, yet seldom positive (12%/42%) in LWD ($P < .001$). Affected joint synovial fluid PCR was frequently positive in both CWD (100%, 3/3) and LWD (85%, 6/7).

Conclusions. These results support the role of small bowel biopsy PAS stain/PCR in the diagnosis of CW, though this approach may be of limited utility in LWD or CNS WD without gastrointestinal symptoms. Affected joint synovial fluid or cerebrospinal fluid PCR was frequently positive in both CWD and LWD, supporting its diagnostic usefulness.

Keywords. diagnostics; PAS; PCR; *Tropheryma whipplei*; Whipple disease.

Whipple disease (WD) is a chronic infection caused by *Tropheryma whipplei* [1]. In 1949, Black-Schaffer first described the classic WD (CWD) histologic finding of Periodic acid-Schiff (PAS)–positive macrophages within the intestinal mucosa and lymph nodes, which was later correlated with the presence of *T. whipplei* bacilli within the macrophage cytoplasm [2, 3]. Subsequently, PAS staining of formalin-fixed paraffin-embedded (FFPE) small bowel (SB) tissue became the standard WD diagnostic test and is commonly followed by amylase or diastase treatment (ie, PAS-D) to remove glycogen to aid in detection of *T. whipplei* bacilli. Since the identification of *T. whipplei* in 1992, polymerase chain reaction (PCR) assays targeting *T. whipplei* have been developed with excellent sensitivity [4–8]. Additional methods include organism cell culture and immunohistochemical staining, although neither is practical or commonly available [9, 10]. Despite these advances, WD remains a diagnostic challenge due to its rarity and variable presentation, resulting in delayed or missed diagnosis [1, 11, 12].

Fewer than 2000 WD cases have been reported in the literature since WD was first described [13]. The majority were classified as CWD, in which nonspecific gastrointestinal manifestations predominate after a period of prodromal joint involvement and constitutional symptoms [1, 11]. CWD frequently involves the nervous system, with 10%–46% of patients developing neurologic symptoms, and less commonly affects the endocardium, uvea, lymphatic system, pulmonary parenchyma, and pleural cavities [13–15]. In contrast, localized WD (LWD) without classic gastrointestinal involvement (including isolated *T. whipplei* endocarditis, polyarticular inflammatory arthritis, or localized neurologic infection) is becoming increasingly recognized, particularly since the advent of *T. whipplei* PCR, which can be performed on a variety of tissues and body fluids [1, 16–20].

The role of PCR in the WD diagnostic paradigm remains unclear. Intestinal tissue PCR has been traditionally ordered as a confirmatory test after PAS staining in CWD cases [11]. Some have recommended PCR in parallel to PAS staining [8, 21]. Individualized diagnostic approaches for localized *T. whipplei* infection have not been fully investigated and thus are likely underutilized. Recent series report SB PAS stain and PCR positivity in only 39%–48% and 55%–93% of CWD cases without typical gastrointestinal symptoms, respectively [11, 16]. *T. whipplei* synovial fluid PCR has been proposed as the

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firstline diagnostic test for seronegative arthritis [17, 22, 23]. Cerebrospinal fluid (CSF) PCR has been recommended when central nervous system (CNS) WD is suspected or in asymptomatic patients, yet its role and usage in WD diagnosis are unclear [11, 24, 25].

We retrospectively investigated diagnostic approaches and methodologies employed at our institution to diagnose WD from 1994 to 2016; 1994 was selected as the start date as *T. whipplei* PCR has been offered routinely at our institution since 1994. We aimed to assess the various testing yields and diagnostic methods employed based on variations in clinical manifestations, comparing classic with nonclassic cases.

METHODS

We retrospectively collected all WD cases diagnosed at the Mayo Clinic between 1994 (when *T. whipplei* PCR became available at our institution) and January 1, 2016. The PCR methodologies used at our institution since 1994 have been previously described [26]. Microbiology laboratory databases were queried for positive *T. whipplei* PCR results performed at our institution during the study period. Anatomic pathology databases were queried for the terms "Whipple disease" AND "positive" AND/OR "consistent." These findings were confirmed against a separate query of the same database for the terms "PAS" OR "PAS-D" AND "positive." Duplicate results, testing ordered by providers external to our institution, and tests to confirm disease relapse were excluded. These queries were confirmed by cross-checking against a query of WD cases in our electronic medical record (EMR). The EMR was reviewed to ascertain WD symptoms, diagnosis, treatment, and response to therapy.

Additional data obtained included demographics, clinical manifestations, medical specialty (initial and diagnosing), laboratory, imaging, and WD diagnostic testing. Diagnostic methodology and test results were stratified by CWD, LWD arthritis, and CNS WD. CWD was defined as systemic disease involvement with prodromal symptoms in addition to multiple-organ involvement of the gastrointestinal system, joints, cardiopulmonary system, lymphatic system, and/or CNS. LWD arthritis and CNS WD were defined as the presence of primarily joint or CNS involvement, respectively, with or without prodromal or constitutional symptoms, with minimal, if any, other organ involvement, particularly gastrointestinal manifestations. Results were analyzed using descriptive statistics and likelihood ratio analysis to compare diagnostic test yield. Standard statistical software (JMP, version 12, SAS Institute, Cary, NC) was used for analysis. This study was approved by the Mayo Clinic Institutional Review Board.

RESULTS

Retrospective database searches yielded 35 cases of WD with 47 positive *T. whipplei* PCR results, 16 positive PAS SB biopsy results, and 2 PAS-positive endocardial tissue biopsies consistent with WD. Two cases (each with 1 single positive blood PCR) were excluded from further analysis because neither patient demonstrated WD symptoms, nor were they treated for WD. Thus, 33 WD cases were diagnosed at our institution from 1994 to January 2016, with 2 being reported previously [27, 28]. Eighteen patients (55%) had CWD, 9 (27%) had LWD arthritis, and 6 (18%) had localized CNS WD.

The cohort consisted of 28 (85%) males with a mean (SD) age of 52 (13) years at the time of diagnosis. Table 1 reports patient characteristics and symptoms by disease manifestation. Diagnosis was confirmed a median (range) of 4.6 (0.6–22.5) years after prodromal symptoms presented. Mean (IQ1, 3) time from initial presentation to our institution to diagnosis was 3 (3, 8.25) months, though diagnosis was delayed more than 1 year after presentation in 5 cases. Table 2 displays initial and confirmatory testing results for each case. Previous misdiagnosis was common, including seronegative inflammatory arthritis (n = 5), rheumatoid arthritis (n = 2), sarcoidosis (n = 2), adult Still disease (n = 1), polymyalgia rheumatica (n = 1), chronic Lyme disease (n = 1), and chronic meningitis (n = 1).

Table 1. Differences in Clinical Data for 33 WD Patients by Whipple Disease Type: Classic vs Localized

Characteristics	Classic WD (n = 18)	Localized WD Arthritis (n = 9)	Localized CNS WD (n = 6)
Male, No. (%)	17 (94)	7 (78%)	4 (67)
Mean age (SD), y	52 (13)	46 (15)	56 (9.3)
Median time from initial symptoms to diagnosis (IQ1,3), y	5.4 (2.6, 6.8)	5.8 (1.4, 6.3)	2.7 (1.5, 3.5)
Previously immunosuppressed, No. (%)	7 (39)	6 (67)	2 (33)
General systemic involvement, No. (%)	18 (100)	4 (44)	5 (83)
GI involvement, No. (%)	16 (89)	0 (0)	1 (16)
Joint involvement, No. (%)	17 (94)	9 (100)	1 (16)
Cardiac involvement, No. (%)	3 (17)	0 (0)	0 (0)
CNS involvement, No. (%)	1 (11)	2 (22)	6 (100)
Anemia, No. (%)	17 (94)	3 (33)	4 (67)
Elevated inflammatory markers, No. (%)	15 (93)	4 (44)	1 (25)
Fat soluble vitamin deficiencies, No. (%)	7 (58)	0 (0)	1 (33)

Abbreviations: CNS, central nervous system; GI, gastrointestinal; WD, Whipple disease.

Table 2. Diagnostic Approach With Initial and Confirmatory Diagnostic Test Results by Whipple Disease Type: Classic vs Localized

				Confirmatory Tests				
Case	WD Type	Initial Diagnostic Test	Initial Test Result	Small Bowel PAS/PCR	Synovial Fluid PCR	CSF PCR	Blood PCR	Other PCR[a]
1	CNS WD	SB PAS/PCR	+/+			+		
2	CWD	SF PCR	+	+/+		-	-	-
3	CWD	SB PAS/PCR	+/+				+	
4	CWD	SB PAS/PCR	+/+				+	
5	CWD	SB PAS/PCR	+/+			+		
6	CWD	SB PAS/PCR	+/+				+	
7	CWD	SB PAS	+					
8	LWD	SB PAS	+					
9	CNS WD	CSF PCR	+	-/+			-	
10	CNS WD	CSF PCR	+	-/-				
11	LWD	SF PCR	+	-/-			-	
12	CNS WD	CSF PCR	+	-/-				
13	LWD	SF PCR	+	-/+				
14	LWD	SF PCR	+				+	
15	CWD	SB PAS/PCR	+/+					
16	LWD	SB PAS/PCR	-/+			-	-	
17	LWD	Blood PCR	+	-/-	+			
18	CNS WD	CSF PCR	+	-/-				
19	CWD	SB PAS/PCR	+/+					
20	CNS WD	SB PAS	-				+	+
21	CWD	SB PAS/PCR	-/-				+	+
22	LWD	SF PCR	+	-/-				
23	CWD	SB PAS/PCR	-/+				+	+
24	LWD	SB PAS/PCR	-/-		+	-	-	
25	CWD	Endocardial biopsy PAS/PCR	+/+	+/+			-	
26	CWD	SB PAS/PCR	+/+					
27	CWD	SF PCR	+				-	
28	LWD	SB PAS/PCR	-/+		-		-	
29	CWD	Endocardial biopsy PAS/PCR	+/+				+	
30	CWD	SB PAS/PCR	+/+			-	-	
31	CWD	SB PAS	+				+	
32	CWD	SB PAS/PCR	+/+					
33	CWD	SF PCR	+			-	-	

+ indicates positive result. – indicates negative result. Blank space indicates test not performed.

Abbreviations: CNS WD, localized *T. whipplei* central nervous system infection; CSF, cerebrospinal fluid; CWD, classic Whipple disease; LWD, localized *T. whipplei* arthritis; PAS, Periodic acid-Schiff; PCR, polymerase chain reaction; SB, small bowel; SF, synovial fluid.

[a]Other PCR includes 2 vitreous aqueous humor fluid PCRs (1 negative and one positive), 1 lymph node tissue specimen PCR (positive), and 1 arterial thrombus surgical pathology specimen PCR (positive).

Furthermore, 43% of patients were prescribed immunosuppressive therapy for misdiagnosis treatment. Chronic therapy for *T. whipplei* included 1 or more of the following: penicillin, tetracycline, doxycycline, ceftriaxone, and trimethoprim/sulfamethoxazole. Patients were followed for a mean (SD) of 10.5 (7.3) years after diagnosis, during which 4 patients had WD relapse and 6 died, all of non-WD-related causes.

Classic Whipple Disease

At the time of diagnosis, all patients with CWD reported systemic symptoms including intermittent fevers, fatigue, chills, and/or night sweats, whereas elevated inflammatory markers and anemia were found in 83% and 94% of cases, respectively. Additionally, 17 of 18 (94%) patients with CWD had prodromal arthralgias for an average of 72 months before presentation, whereas 89% had nonspecific gastrointestinal symptoms, including intermittent abdominal pain, chronic diarrhea, and/or weight loss. Five patients with CWD had developed neurologic manifestations, including cognitive impairment, psychiatric changes, and/or movement abnormalities. Lymphadenopathy was present, clinically or on radiographic imaging, in 7 CWD cases. Additional manifestations among CWD patients included endocarditis (n = 2), pericarditis (n = 1), arterial thrombus (n = 1), and fat-soluble vitamin deficiencies (n = 7).

The initial diagnostic test obtained was SB biopsy with PAS staining in 13 of 18 CWD cases with 11 (85%) positive results

(Table 2); 7/12 (63%) of the biopsies were obtained from the duodenum while rest were from the jejunum, stomach, mesenteric lymph nodes, lymph nodes, parietal pericardium, terminal ileum, or colon, with tissue obtained from multiple locations in some patients. Both negative results were in the setting of antibiotics for more than 30 days before biopsy, prescribed for presumed chronic Lyme disease and *Pneumocystis jirovecii* pneumonia prophylaxis for chronic immunosuppression for presumed seronegative arthritis. Duodenal PCR was ordered in parallel to PAS stain in 11 cases (10 were positive and 1 negative in a case with a negative PAS stain). The 2 patients with negative duodenal PAS stains were diagnosed by PCR (case 1: blood and lymph node tissue specimen; case 2: blood and arterial thrombus). Additional diagnostic tests ordered in these 13 cases included 1 negative vitreous aqueous humor PCR and 9 blood PCRs (3 positive). CSF PCR was performed in 4 of the 5 patients with neurologic manifestations (1 positive).

Five patients with CWD did not undergo SB sampling as the initial diagnostic test. Two patients with endocarditis were diagnosed with positive endocardial tissue PAS stain and PCR. One case was confirmed with blood PCR; the other case was confirmed with positive duodenal PAS stain, and PCR after blood PCR was negative. The other 3 patients were diagnosed after undergoing arthrocentesis with positive synovial fluid PCR. One of these 3 subsequently underwent confirmatory SB testing with positive duodenal PAS stain and PCR.

Localized *T. whipplei* Arthritis

Nine patients with localized *T. whipplei* arthritis reported arthralgias for a median (range) of 5.0 (1.0–22) years before presentation to our institution. None reported gastrointestinal symptoms (abdominal pain, chronic diarrhea, or weight loss), whereas 2 reported minor cognitive changes (memory loss). Four had intermittent systemic symptoms of fatigue, night sweats, and/or asthenia. None reported weight loss or developed vitamin deficiencies, but elevated inflammatory markers were present in 4.

In 4 of the 9 LWD cases, the initial diagnostic test was synovial fluid PCR with 4/4 positive (Table 2). Small bowel PAS stain and PCR were ordered to confirm the initial result in 3 of these 4 cases (1/3 PCR and 0/3 PAS stain positive). Four patients underwent initial testing with SB PAS stain; PCR was ordered in parallel to PAS in 3 of 4 cases. Results were PAS (without PCR) positive in 1 case, negative PAS/positive PCR in 2 cases, and negative PCR/negative PAS in 1 case (diagnosis subsequently made with positive synovial fluid PCR). One patient initially diagnosed with blood PCR was confirmed by positive synovial fluid PCR. CSF PCR was negative in the 2 patients reporting cognitive deficits. Synovial fluid total nucleated cell count ranged from 208 to 45 708 cells/mcL (median, 3016 cells/mcL), with the polymorphonuclear cell percentage ranging from 13% to 86% (median, 75%).

Localized *T. whipplei* CNS Infection

Six patients with localized CNS WD presented with cognitive deficits, psychiatric symptoms, movement abnormalities, and/or supranuclear ophthalmoplegia. Uveitis was present in 1 case [28]. Only 1 patient had gastrointestinal symptoms (abdominal pain); none had diarrhea or weight loss. Systemic symptoms including fever, asthenia, fatigue, and chills were present in 5 cases. Laboratory abnormalities included anemia (n = 4), elevated inflammatory markers (n = 1), and vitamin B12 deficiency (n = 1). Residual neurologic deficits were noted in 3 cases after treatment, with 1 relapse.

The initial diagnostic test was lumbar puncture for CSF PCR in 4 of 6 cases, with 4/4 positive (Table 2). Confirmation of diagnosis was pursued in all 4 cases with SB PAS stain/PCR (1/4 PCR and 0/4 PAS stain positive). SB biopsy was the initial diagnostic test in the other 2 CNS WD cases. In 1 case, duodenal PAS was nonreactive (PCR not performed), but blood and vitreous aqueous humor fluid PCR were positive; CSF was not tested. In the second case, duodenal PAS/PCR were positive on initial testing, and subsequently CSF PCR was positive.

Diagnostic Approach and Comparison of Results

The 33 WD patients initially presented to 11 different medical specialties: rheumatology (n = 8), neurology (n = 7), gastroenterology (n = 5), hematology (n = 4), general medicine (n = 4), cardiology (n = 1), ophthalmology (n = 1), pulmonology (n = 1), infectious disease (n = 1), and endocrinology (n = 1) (Figure 1). When initial presentation was to rheumatology, neurology, or gastroenterology, the diagnosis was made by that specialist in 19 of 20 cases (Figure 2). In these 20 cases, consultation from a second specialist was requested in 8 cases (gastroenterology [n = 3], rheumatology [n = 2], infectious disease [n = 2], and neurology [n = 1]). In the other 13 cases, consultation from a gastroenterologist or rheumatologist was requested in 11 cases.

Small bowel testing with PAS stain and/or PCR was ordered as the initial WD test in 18 of 33 (55%) cases but ordered by

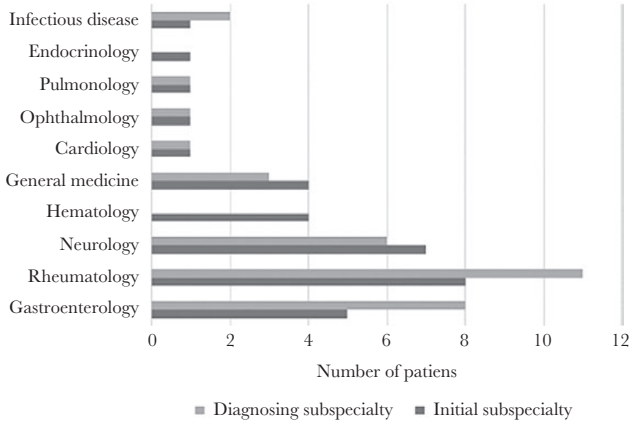

Figure 1. Proportion of medical subspecialties that initially evaluated and ultimately diagnosed 33 patients with Whipple disease.

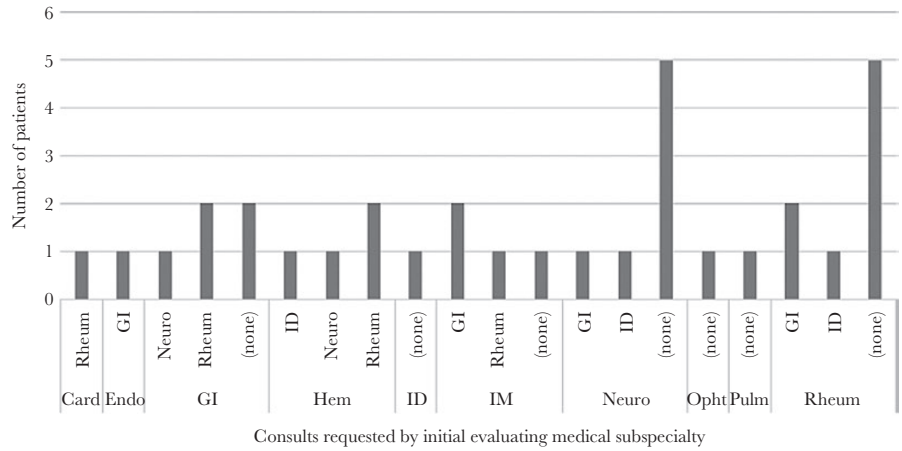

Figure 2. Proportion of medical subspecialties consulted by initially evaluating subspecialty in 33 patients with Whipple disease. Abbreviations: Card, cardiology; Endo, endocrinology; GI, gastroenterology; Hem, hematology; ID, infectious diseases; IM, internal medicine; Neuro, neurology; Opht, ophthalmology; Pulm, pulmonology; Rheum, rheumatology.

a gastroenterologist in 4 (22%) cases. When CSF or synovial fluid PCR was obtained as the initial WD test, these diagnostics were almost exclusively (10/11 cases) ordered by a neurologist or rheumatologist, respectively. Confirmatory testing with a different method was pursued in 31 of the 33 cases with a 64% positive rate.

Differences in Diagnostic Test Results

Table 3 reports diagnostic test yields for CWD, LWD, and CNS WD. Duodenal PAS stain was overwhelmingly positive (86%) in the 18 CWD cases; however, it was rarely positive (13%) in the 15 non-CWD cases with minimal, if any, gastrointestinal manifestations ($P < .001$). Small bowel PCR was positive in 42% (5/12) of localized WD cases without diarrhea compared with 13% PAS stain reactivity ($P = .018$). Duodenal PAS stain reactivity was similar in LWD arthritis (12%, 1/8) and CNS WD cases (17%, 1/6). Similarly, duodenal PCR positivity rates were comparable in LWD (42%, 3/7) and CNS WD cases (40%, 2/5).

Synovial fluid PCR testing was high yield, with positive results in 89% (8/9) of patients with arthritis. Synovial fluid PCR positivity rates were comparable in patients with CWD (100%, 3/3) and localized *T. whipplei* arthritis (83%, 5/6; $P = .35$). CSF PCR was positive in 100% (5/5) of CNS WD cases, whereas it was only positive in 25% (1/4) of CWD patients with neurologic symptoms ($P = .009$). CSF PCR was negative in patients with localized *T. whipplei* arthritis with cognitive changes. Blood PCR lacked sensitivity in CWD and LWD cases (CWD: 54%, 6/11, including 50%, 1/2, positivity in 2 cases of endocarditis; *T. whipplei* arthritis: 33%, 2/6; CNS WD: 50%, 1/2; $P = .69$).

DISCUSSION

We report 33 WD cases diagnosed at our institution from 1994 to 2016, which is to our knowledge the largest series of American WD cases reported since the development of *T. whipplei* PCR. Eighteen patients (55%) were diagnosed with CWD, 9 (27%) with localized WD arthritis, and 6 (18%) with localized CNS WD. These results support the role of PAS stain and PCR of duodenal tissue in the diagnosis of CWD. Although 5 patients did not have histological findings on small-bowel biopsies or did not have a biopsy done at all, their symptoms and PCR positivity from other sites were consistent with CWD. This is consistent

Table 3. Diagnostic Test Yields Differ by Classic vs Localized Whipple Disease

	Classic WD (n = 18), No. Positive/ No. Tested (%)	Localized WD Arthritis (n = 9), No. Positive/No. Tested (%)	Localized CNS WD (n = 6), No. Positive/No. Tested (%)	*P* Value
Small bowel biopsy PAS stain[a]	13/15 (86)	1/8 (12)	1/6 (17)	<.001*
Small bowel biopsy PCR	12/13 (92)	3/7 (42)	2/5 (40)	.018*
Synovial fluid PCR	3/3 (100)	6/7 (85)	0/0 (0)	.35
Cerebrospinal fluid PCR	1/4 (25)	0/2 (0)	5/5 (100)	.009*
Blood PCR	7/12 (58)	2/6 (33)	1/2 (50)	.69
Other PCR[a]	4/5 (80)	0/0 (0)	1/1 (100)	.52

Abbreviations: CNS, central nervous system; PAS, Periodic acid-Schiff; PCR, polymerase chain reaction; WD, Whipple disease.

*P value considered significant if <.05.

[a]Other PCR included 2 vitreous aqueous humor fluid PCRs (1 negative and 1 positive), 1 lymph node tissue specimen PCR (positive), and 1 arterial thrombus surgical pathology specimen PCR (positive).

with findings in the largest series of CWD, which showed that in 91% of 191 cases of CWD, the duodenum had characteristic histological changes in SB biopsies [11]. However, the diagnostic yield of SB PAS stain, and PCR to a lesser degree, was decreased in non-CWD cases without gastrointestinal involvement. Diagnosis in non-CWD was most often made by positive PCR of blood, CSF, synovial fluid, or other tissue, suggesting that a combination of clinical suspicion for WD and sampling of potentially affected sites is helpful in diagnosis even in the absence of SB PAS stain or PCR positivity. Affected joint synovial fluid PCR was frequently positive in both CWD and LWD. Blood PCR was poorly sensitive in all WD cases, irrespective of classical or local disease involvement, including endocarditis. There were 2 apparently false-positive blood PCR results in this study. Stool and saliva PCR were not performed at the Mayo Clinic as a routine clinical test during the study period.

This cohort includes a significant proportion (45%) of cases with LWD, which is greater than the proportion reported in European studies. Gunther et al. reported only CWD cases, with few nonintestinal PCR tests, whereas Lagier and colleagues reported 80% CWD cases and 20% isolated WD cases (mostly endocarditis) in their series [1, 11]. Endocarditis is the most common LWD manifestation reported in European studies, though multiple series of localized *T. whipplei* arthritis have also been reported recently [1, 16, 17, 29]. Fleming and colleagues described 29 WD cases diagnosed at our institution from 1954 to 1984 [12]. In their publication, 12 (43%) patients developed CNS involvement, nearly double the proportion in recent European series [1, 11, 13]. The etiology of these differences is unclear but could be due to referral bias or geographic variance, possibly related to varying bacterium strains. Geographic variance has recently been reported [16].

In our cohort, SB PAS stain and PCR were positive in 14% and 42%, respectively, of LWD cases without weight loss or chronic diarrhea. These results are congruent with previous studies demonstrating increased sensitivity of duodenal PCR compared with PAS staining [11, 16, 30]. Lagier and colleagues recently reported 24 cases of LWD in which duodenal tissue PAS stain and PCR were positive, 0% (0/22) and 6.7% (1/15), respectively [1].

Localized *T. whipplei* arthritis and localized CNS WD are most commonly diagnosed by synovial fluid and CSF PCR, respectively, as shown in our cohort and others [1, 16, 17]. These findings support the role of nonintestinal PCR testing as a preferred initial diagnostic investigation in cases of non-CWD.

Due to the rarity and variable presentation of WD, misdiagnosis and delay in correct diagnosis is commonplace, and patients may be referred to multiple different medical subspecialties before diagnosis. The 33 WD patients presented to 11 different specialties. Most patients initially presented to a gastroenterologist, rheumatologist, or neurologist. Small bowel–based testing was ordered commonly by all specialists, regardless of subspecialty; however, nearly all PCR tests of synovial fluid or CSF fluid were ordered by a rheumatologist or neurologist, respectively. With the increased awareness of the diagnostic value of WD PCR testing, we expect an increase in familiarity of synovial fluid– and CSF fluid–based *T. whipplei* PCR testing. It is difficult to develop a 1-size-fits-all diagnostic scheme for WD; however, this study supports the use of extra-intestinal PCR testing and early referral to gastroenterology, rheumatology, or neurology to mitigate delay in diagnosis.

Potential limitations exist in our study. Physician practices could have been influenced by progression in medical knowledge over 2 and a half decades, thus potentially affecting diagnostic paradigms. Temporal trends of diagnostic methodologies were not assessed but could be evaluated in future investigations. Although the PCR assay evolved from a conventional PCR assay to a real-time PCR assay over the study period, clinical performance characteristics did not change. Similarly, PAS staining on FFPE tissue remained unchanged during this time frame.

In conclusion, this study reports the test yield and diagnostic paradigms employed by physicians at our institution for WD diagnosis over 22 years, during which WD PCR was routinely available. Yield of SB PAS and PCR was low in patients with WD without overt gastrointestinal symptoms. Synovial fluid or CSF PCR should be strongly considered during the initial diagnostic investigation in patients presenting with localized symptoms consistent with localized *T. whipplei* arthritis or localized CNS WD, respectively.

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Author contributions. Nicholas R. Crews contributed to study concept and design; acquisition of data; analysis and interpretation of data; and drafted the manuscript. Kelly A. Cawcutt contributed to study concept and design; acquisition of data; analysis and interpretation of data. Bobbi S. Pritt contributed to acquisition of data and critical revision of the manuscript for important intellectual content. Robin Patel contributed to acquisition of data and critical revision of the manuscript for important intellectual content. Abinash Virk contributed to study concept and design; acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; study supervision.

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